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# Ultrastructure of *Besnoitia besnoiti* Tissue Cysts and Bradyzoites

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ABSTRACT. *Besnoitia besnoiti* is an economically important tissue cyst-forming apicomplexan of cattle in Africa and Israel. Tissue cysts and bradyzoites of *B. besnoiti* from the skin of a naturally infected bull were studied by transmission electron microscopy. Tissue cysts enclosed host cell and bradyzoites. Bradyzoites were 6–7.5 × 1.9–2.3 μm in size and contained organelles found in coccidian merozoites including numerous micronemes, rhoptries, amylopectin granules, and a posteriorly located nucleus. Enigmatic bodies, characteristically found in *Besnoitia* sp. bradyzoites, were not observed. Therefore, enigmatic bodies should be removed as a generic character of the bradyzoites of *Besnoitia* species.

**Key Words.** Cattle, enigmatic bodies, ultrastructure.

*BESNOITIA* spp. are coccidians affecting livestock and wild animals (Dubey et al. 2002; Frenkel 1977; Leighton and Gajadhar 2001; Paperna and Lainson 2001). There are eight named species of *Besnoitia*: *B. bennetti* Babudieri 1932; *B. besnoiti* (Marotel 1912) Henry 1913; *B. caprae* Njenga, Bwangamoi, Mutiga, Kangethe and Mugera 1993; *B. darlingi* (Brumpt 1913) Mandour 1965; *B. jellisoni* Frenkel 1953; *B. wallacei* (Tadros and Laarman 1976) Frenkel 1977; *B. tarandi* (Hadwen 1922) Levine 1961, and *B. oryctofelisi* Dubey, Sreekumar, Lindsay, Hill, Rosenthal, Venturini, Venturini and Greiner 2003. The life cycle is known for only *B. wallacei*, *B. darlingi*, and *B. oryctofelisi* for all of which cats are the definitive hosts (Dubey et al. 2002, 2003; Smith and Frenkel 1977, 1984; Wallace and Frenkel 1975). Tachyzoites and tissue cysts are stages of *Besnoitia* found in intermediate hosts and schizonts and gamonts occur in the definitive host.

*Besnoitia* tissue cysts are distinct from other tissue-cyst forming coccidia because of the inclusion of the host cell nucleus or nuclei within the tissue cyst wall of *Besnoitia* (Sheffield 1968). Diagnosis based on morphology of *Besnoitia* is complicated by observed variation in size of tissue cysts and the thickness of the cyst wall, features that depend on the host, the site parasitized, tissue affinity, and duration of infection. For example, Paperna and Lainson (2001) found differences between tissue cysts of the same *Besnoitia* sp. in lizards and mice. At present there are no known differences among bradyzoites of *B. jellisoni* (Mehlhorn et al. 1974; Scholtyseck et al. 1973; Senaud 1969; Senaud et al. 1972, 1974; Sheffield 1968), *B. besnoiti* (Shkap et al. 1988), *B. tarandi* (Hillali et al. 1990), *B. darlingi* (Dubey et al. 2002), *B. oryctofelisi* (Dubey and Lindsay 2003), *B. caprae* (Heydorn et al. 1984; Njenga et al. 1995), or *Besnoitia* sp. as described by Paperna and Lainson (2001), although a critical comparison has not been made. Bradyzoites of *Besnoitia* species have characteristic enigmatic bodies, first reported by Senaud (1969) in the perinuclear region of *B. jellisoni*.

*Besnoitia besnoiti* is an economically important parasite of cattle in Africa, Israel, and other countries (Bigalke 1968; Levine 1973). It also infects goats and wild ruminants. A similar parasite has been described from goats from Africa and this parasite has been called *B. caprae*. However, genetically *B. caprae* and *B. besnoiti* are identical (Ellis et al. 2000). Njenga et al. (1995) described ultrastructural differences between bradyzoites of *B. caprae* and *B. besnoiti* but review of their data does not support this distinction. In the present paper we describe the ultrastructure of *B. besnoiti* tissue cysts and bradyzoites and report the absence of enigmatic bodies in its bradyzoites.

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## MATERIALS AND METHODS

Subcutaneous and cutaneous tissue from a bull (*Bos taurus*) with chronic besnoitiosis in Israel were fixed in cold 0.5% (v/v) glutaraldehyde, 3% (v/v) paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.2 for 1 h. Then the pellets were washed in 0.1 M sodium cacodylate and left at 4 °C overnight. After that 1% (w/v) osmium tetroxide in the same buffer was added for up to 4–5 h, depending on its size, until the pellet became black. The material was then washed four times with 0.1 M cacodylate and transferred to Beltsville, MD. For transmission electron microscopy (TEM), tissues were processed as described by Fritz et al. (1999).

## RESULTS

The *Besnoitia* tissue cyst wall enclosed the host cell and bradyzoites (Fig. 1). The innermost cyst wall layer, the parasitophorous vacuolar membrane (pvm), was thin and lined by a lucent granular layer, approximately 0.2 μm thick (Fig. 1, 2). Several host cell nuclei were enclosed in the layer above the pvm. The outermost layer (i.e. secondary cyst wall described by Heydorn et al. 1984) surrounded the host cell (Fig. 1). Bradyzoites were located in the parasitophorous vacuole (pv) that contained degenerated parasites, vacuolated, and tubular structures (Fig. 2).

Bradyzoites were numerous and contained organelles most commonly found in coccidian bradyzoites, namely the conoid, rhoptries, micronemes, dense granules, mitochondrion, nucleus, lipid bodies, and amylopectin granules. Five longitudinally cut bradyzoites were 6.0–7.5 × 1.9–2.3 μm in size (Fig. 3). Numerous micronemes extended up to the posterior (non-conoidal) end but most were at the anterior (conoidal) end and often arranged in rows. Up to 70 micronemes were present in one plane of section. Rhoptries had a long neck and a bulbous end approximately 0.2 μm in diameter. Up to eight, electron-dense rhoptries were present in some bradyzoites although most sections had only one or two (Fig. 3). A mitochondrion-like structure was located anterior to the nucleus. The nucleus was located in the posterior half of the bradyzoite and occupied nearly half of the bradyzoite width. Numerous amylopectin granules were present, mostly posterior to the nucleus. A few dense granules and lipid bodies were also present. Enigmatic bodies were not observed in over 100 bradyzoites.

## DISCUSSION

In the present study enigmatic bodies were not found in bradyzoites of *B. besnoitia*. Shkap et al. (1988) briefly reported on the ultrastructure of *B. besnoiti* bradyzoites but did not mention the presence or absence of enigmatic bodies. These enigmatic bodies were also not mentioned in bradyzoites of *Besnoitia* sp. from goats (Heydorn et al. 1984) and in *B. caprae* (Njenga et


Fig. **1**. Transmission electron micrograph of a tissue cyst of *Besnoitia besnoiti*. Note secondary cyst wall (SCW) enclosing host cell (HC), host cell nuclei (HCN), parasitophorous vacuolar membrane (PVM), and numerous bradyzoites (BZ).

al. 1995). Enigmatic bodies are present in bradyzoites of *B. darlingi* (Dubey et al. 2002), *B. tarandi* (Hilali et al. 1990), *Besnoitia* sp. described by Paperna and Lainson (2001), and *B. oryctofelisi* (Dubey and Lindsay 2003), but absent in *B. bennetti* (van Heerden et al. 1993). Njenga et al. (1995) described membrane-bound wall-forming bodies (wb) in bradyzoites of *B. besnoiti* and *B. caprae*; these bodies had not been previously reported in coccidian merozoites. The wb were reported to be

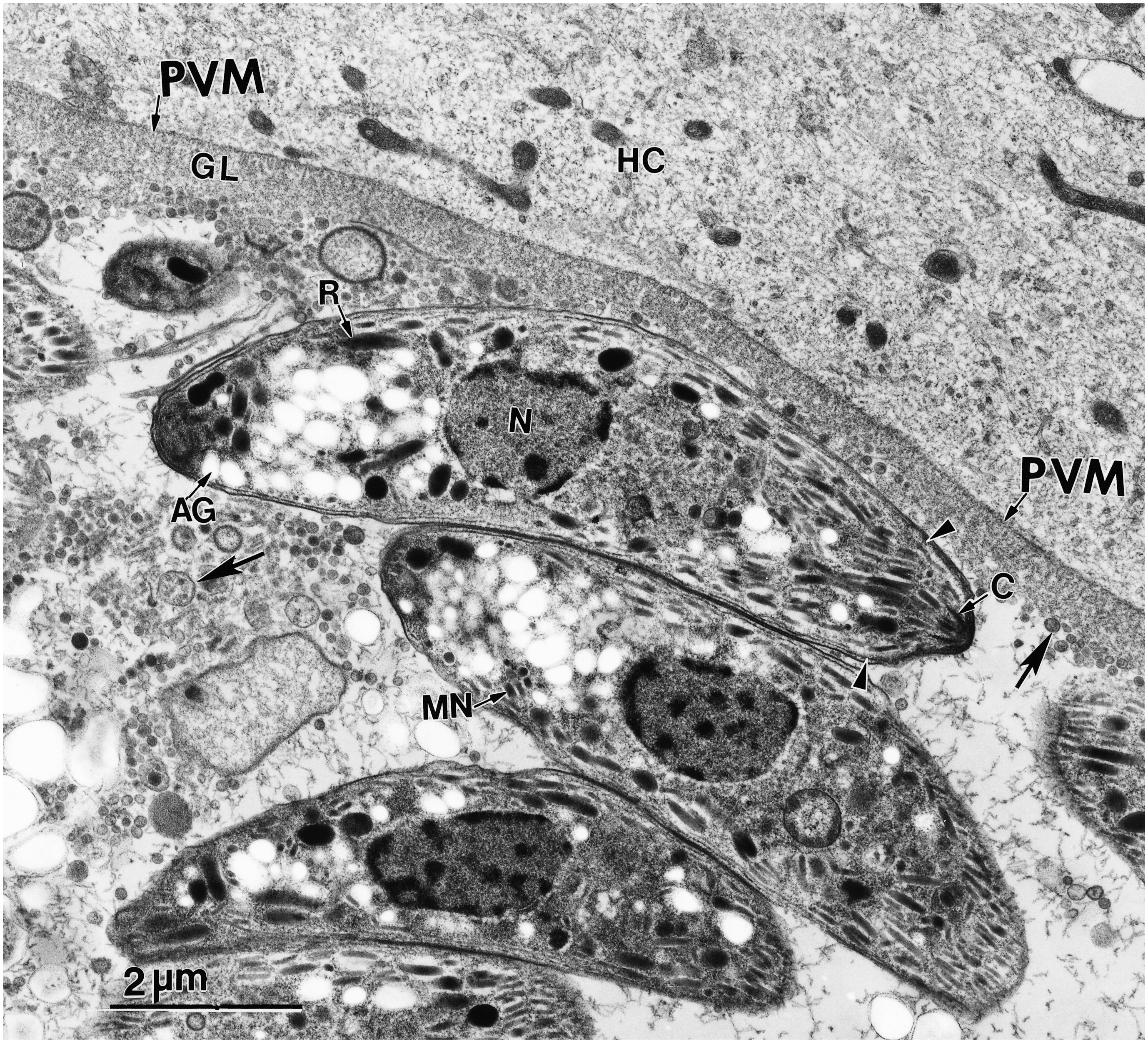


Fig. **2**. Transmission electron micrograph of tissue cyst wall of *Besnoitia besnoiti*. The parasitophorous vacuolar membrane (PVM) is lined by a granular layer (GL), and a longitudinally cut bradyzoite is butted against the granular layer. Note numerous micronemes (MN), a few rhoptries (R), a nucleus (N), and amylopectin granules (AG) and host cell (HC). Vacuolated structures and degenerated parasites (arrows) are present in the parasitophorous vacuole. There is a slight deposit of subpellicular granular material (arrowheads) at the conoidal (C) end.

round, dark, dense, and homogenous and found anterior to the nucleus in *B. caprae* bradyzoites. In *B. besnoiti* bradyzoites, these wb were reported to contain several smaller membrane-bound bodies and some were dividing (Njenga et al. 1995). Membrane-bound bodies were also seen in *B. besnoiti* bradyzoites in the present study but these were probably sections of the long mitochondrion (see Fig. 3). Njenga et al. (1995) equated their wb in *B. caprae* and *B. besnoiti* bradyzoites to wall-forming bodies in macrogamonts of coccidia, which is an error. In all coccidians, wall-forming bodies are found only in macrogamonts and give rise to oocyst walls. Although we have not studied *B. caprae* bradyzoites, a critical review of results reported by Njenga et al. (1995) indicates no valid structural differences between *B. caprae* and *B. besnoiti* bradyzoites. This conclusion is supported by molecular results reported by Ellis et al. (2000).

## LITERATURE CITED

Bigalke, R. D. 1968. New concepts on the epidemiological features of bovine besnoitiosis as determined by laboratory and field investigations. *Onderstepoort J. Vet. Res.*, **35**:3–138.

Dubey, J. P. & Lindsay, D. S. 2003. Development and ultrastructure of *Besnoitia oryctofelisi* tachyzoites, tissue cysts, bradyzoites, schizonts and merozoites. *Int. J. Parasitol.*, In press.

Dubey, J. P., Sreekumar, C., Lindsay, D. S., Hill, D., Rosenthal, B. M., Venturini, L., Venturini, M. C. & Greiner, E. C. 2003. *Besnoitia*

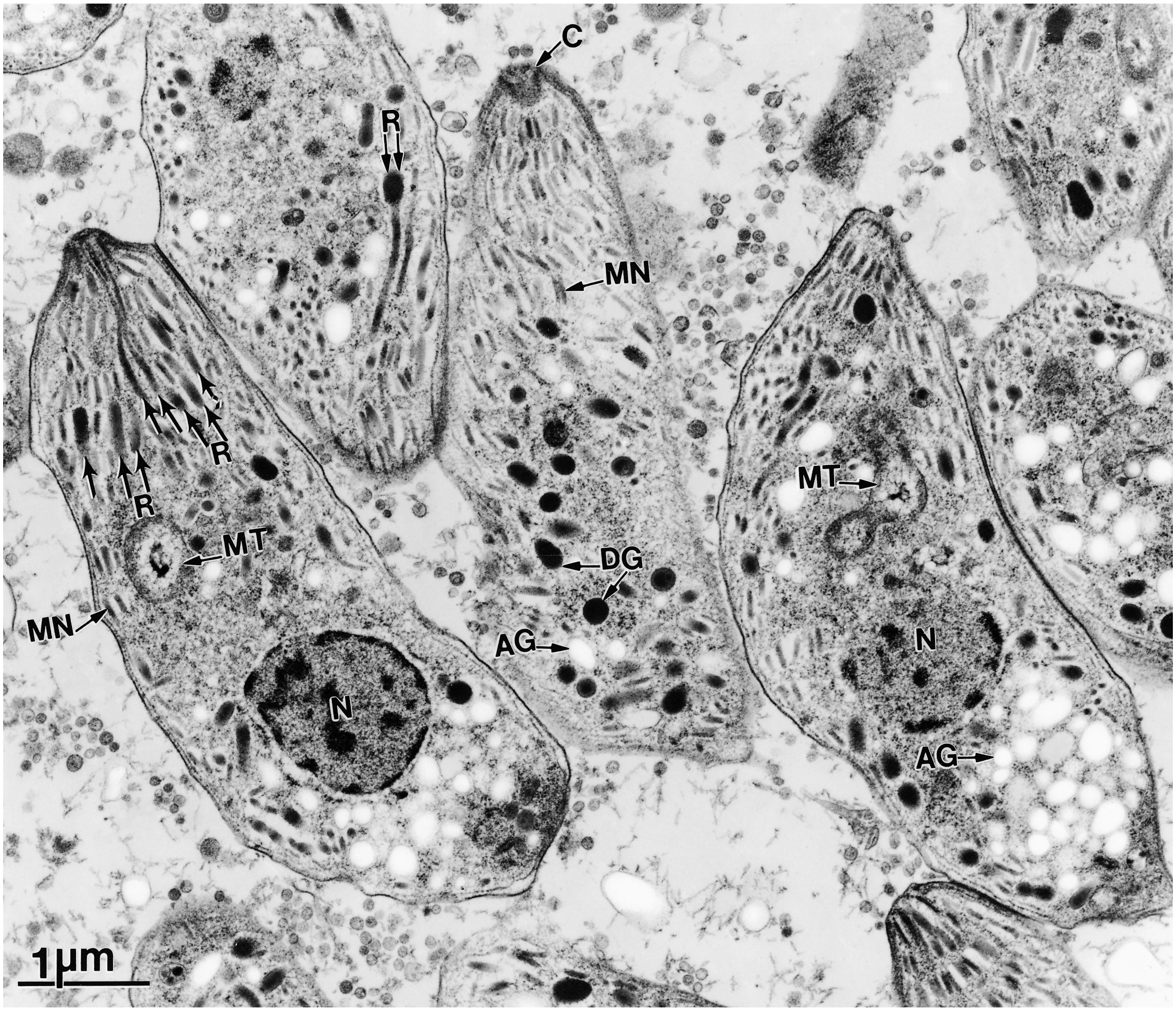


Fig. **3**. Transmission electron micrograph of bradyzoites of *Besnoitia besnoiti*. Note numerous micronemes (MN) at the conoidal end (C), up to eight rhoptries (R) in one plane of section in the bradyzoite on extreme left side, short bulbous end of a rhoptrie (R with double arrows), a posteriorly located nucleus (N), a mitochondrion (MT) like structure, amylopectin granules (AG) concentrated at the non-conoidal end, and dense granules (DG).


*oryctofelisi* n. sp. (Protozoa: Apicomplexa) from domestic rabbits. *Parasitology*, **126**:526–539.

Dubey, J. P., Lindsay, D. S., Rosenthal, B. M., Sreekumar, C., Hill, D. E., Shen, S. K., Kwok, O. C. H., Rickard, L. G., Black, S. S. & Rashmir-Raven, A. 2002. Establishment of *Besnoitia darlingi* from opossums (*Didelphis virginiana*) in experimental intermediate and definitive hosts, propagation in cell culture, and description of ultrastructural and genetic characteristics. *Int. J. Parasitol.,* **32**:1053–1064.

Ellis, J. T., Holmdahl, O. J. M., Ryce, C., Njenga, J. M., Harper, P. A. W. & Morrison, D. A. 2000. Molecular phylogeny of *Besnoitia* and the genetic relationships among *Besnoitia* of cattle, wildebeest, and goats. *Protistologica*, **151**:329–336.

Frenkel, J. K. 1977. *Besnoitia wallacei* of cats and rodents with a reclassification of other cyst-forming isosporoid coccidia. *J. Parasitol.*, **63**:611–628.

Fritz, D. L., Vogel, P., Brown, D. R. & Waag, D. M. 1999. The hamster model of intraperitoneal *Burkholderia mallei* (glanders). *Vet. Pathol.*, **36**:276–291.

Heydorn, A. O., Senaud, J., Mehlhorn, H. & Heinonen, R. 1984. *Besnoitia* sp. from goats in Kenya. *Zeits. Parasitenk.*, **70**:709–713.

Hilali, M., Rehbinder, C. & Nikkilä, T. 1990. Ultrastructure of *Besnoitia* cysts from reindeer (*Rangifer tarandus tarandus* L). *Rangifer*, Special Issue No. 3:335–342.

Leighton, F. A. & Gajadhar, A. A. 2001. *Besnoitia* spp. and besnoitiosis. *In*: Samuel, W. M., Pybus, M. J. & Kocan, A. A., (ed.), Parasitic Diseases of Wild Mammals. 2nd ed., Iowa State University Press, Ames, Iowa. p. 468–478.

Levine, N. D. 1973. Protozoan Parasites of Domestic Animals and of Man. 2nd ed., Burgess Publishing Co., Minneapolis, Minnesota.

Mehlhorn, H., Senaud, J. & Scholtyseck, E. 1974. Ètude ultrastructurale des coccidies formant des kystes: *Toxoplasma gondii*, *Sarcocystis tenella*, *Besnoitia jellisoni* et *Frenkelia* sp.: distribution de la phosphatase acide et des polysaccharides au niveau des ultrastructures chez le parasite et chez l'hote. *Protistologica*, **10**:21–42.

Njenga, J. M., Bwangamoi, O., Kangethe, E. K., Mugera, G. M. & Mutiga, E. R. 1995. Comparative ultrastructural studies on *Besnoitia besnoiti* and *Besnoitia caprae*. *Vet. Res. Commun.*, **19**:295–308.

Paperna, I. & Lainson, R. 2001. Light microscopical structure and ultrastructure of a *Besnoitia* sp. in the naturally infected lizard *Ameiva ameiva* (Teiidae) from north Brazil, and in experimentally infected mice. *Parasitology,* **123**:247–255.

Scholtyseck, E., Mehlhorn, H. & Müller, B. E. G. 1973. Identifikation von Merozoiten der vier cystenbildenden Coccidien (*Sarcocystis, Toxoplasma, Besnoitia, Frenkelia*) auf Grund feinstruktureller Kriterien. *Zeits. Parasitenk.,* **42**:185–206.

Senaud, J. 1969. Ultrastructure des formations kystiques de *Besnoitia jellisoni* (Frenkel 1953) protozoaire, Toxoplasmea, parasite de la souris (*Mus musculus*). *Protistologica,* **5**:413–430.

Senaud, J., Mehlhorn, H. & Scholtyseck, E. 1972. Cytochemische Üntersuchungen an Cystenstadien von *Besnoitia jellisoni*. Elektronenmikroskopischer Nachweis von saurer Phosphatase und Polysacchariden. *Zeits. Parasitenk.,* **40**:165–176.

Senaud, J., Mehlhorn, H. & Scholtyseck, E. 1974. *Besnoitia jellisoni* in macrophages and cysts from experimentally infected laboratory mice. *J. Protozool.,* **21**:715–720.

Sheffield, H. G. 1968. Observations on the fine structure of the ''cyst stage'' of *Besnoitia jellisoni*. *J. Protozool.,* **15**:685–693.

Shkap, V., Yakobson, B. A. & Pipano, E. 1988. Transmission and scanning electron microscopy of *Besnoitia besnoiti*. *Int. J. Parasitol.,* **18**:761–766.

Smith, D. D. & Frenkel, J. K. 1977. *Besnoitia darlingi* (Protozoa: Toxoplasmatinae): cyclic transmission by cats. *J. Parasitol.,* **63**:1066–1071.

Smith, D. D. & Frenkel, J. K. 1984. *Besnoitia darlingi* (Apicomplexa, Sarcocystidae, Toxoplasmatinae): transmission between opossums and cats. *J. Protozool.,* **31**:584–587.

van Heerden, J., Els, H. J., Raubenheimer, E. J. & Williams, J. H. 1993. Besnoitiosis in a horse. *J. S. Afr. Vet. Assoc.,* **64**:92–95.

Wallace, G. D. & Frenkel, J. K. 1975. *Besnoitia* species (Protozoa, Sporozoa, Toxoplasmatidae): recognition of cyclic transmission by cats. *Science,* **188**:369–371.

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